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(54) PROCESS FOR PRODUCING MODIFIED KERATIN PROTEIN

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SPECIFICATION

1. Title Of The Invention

PROCESS FOR PRODUCING MODIFIED KERATIN PROTEIN

2. Claims

1. A process for producing modified keratin protein, in which a keratinous substance is subjected to reduction treatment using a reducing agent in water or a mixed solvent made up of water and a hydrophilic organic solvent to turn disulfide bonds into sulfhydryl groups and is subsequently reacted with a cationizing agent having quaternary nitrogen and groups represented by [*See first two structures on page 205 – trans.*] or CH_2=CH– in one molecule.
2. The production process as set forth in claim 1, in which the keratinous substance is wool, feather, hair, nail, or horn.
3. The production process as set forth in claim 1, in which the hydrophilic solvent is methanol, ethanol, *n*-propanol, isopropanol, acetone, methyl ethyl ketone, dioxane, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, or hexamethylphosphoric triamide.
4. The production process as set forth in claim 1, in which the reducing agent is 2-mercaptoethanol, thioglycolic acid, tri-*n*-butylphosphine, triphenylphosphine, ascorbic acid, sodium hydrogensulfite, or sodium sulfite.

3. Detailed Explanation Of The Invention

The present invention relates to a process for producing modified keratin protein, and, more specifically, to a process for producing a modified keratin protein extremely soluble in water or a mixed solvent made up of water and a hydrophilic organic solvent, where the process is characterized in that, in order to recover protein from a keratinous substance, reduction treatment is carried out in water or in a mixed solvent made up of water and a hydrophilic organic solvent to cleave disulfide bonds (–S–S–) in keratin and produce sulfhydryl groups (–SH), and a quaternary ammonium salt

type cationizing agent is added to the resulting sulfhydryl groups, or said sulfhydryl groups and other functional groups in the protein, for example, hydroxyl, amino, carboxyl, and other functional groups.

One of the methods commonly used in the past for extracting protein from keratinous substances consisted in solubilizing the protein by cleaving the disulfide bonds in keratin by reduction or oxidation treatment. However, the use of copious amounts of various denaturants as solubilization adjuvants in the reduction treatment method makes the procedure extremely burdensome and complicated. For instance, in the reduction and solubilization treatment method based on the use of 2-mercaptoethanol, a reduction reaction is carried out in a strong aqueous solution of urea serving as a solubilization adjuvant, and after the reduction-treated keratin dissolves in the strong aqueous solution of urea, iodoacetic acid is added to sulfhydryl groups, which are unstable to oxidation, and a carboxymethylation reaction, etc. is carried out to produce a stable form of the protein. After that, the undissolved matter is partially removed by filtration, and low molecular impurities, mainly the considerable amount of urea present in the solution of protein in the aqueous urea solution, are removed from the aqueous solution using operations such as dialysis etc. Protein is recovered from the thus obtained aqueous solution of protein by isoelectric precipitation or lyophilization. On the other hand, there is a method, in which protein is recovered from keratinous substances by oxidation, which cleaves the disulfide bonds (–S–S–) to give sulfonic acid groups ($-SO_3H$). It is known, however, that oxidation using peracetic acid brings about hydrolysis of polypeptide bonds in the principal chain, which makes it undesirable as a method for recovering high molecular weight keratin protein.

In addition, the term "protein" used in the present invention refers not to low molecular weight oligopeptides with a typical molecular weight of several thousand or less obtained by hydrolyzing polypeptide bonds in the principal chain of the keratinous substance with acids, alkalis, or oxygen, etc., but mainly to protein with a molecular weight of at least 10,000 or more, preferably, with a molecular weight of 20,000 to 50,000, which is obtained by cleaving the disulfide bonds of the keratinous substance.

The thus obtained protein is supposed to be soluble, but because of the hydrogen bonds, ionic bonds, hydrophobic bonds, etc. in the protein, it is poorly soluble in water and organic polar solvents, and even when it is dissolved, only very weak solutions can be obtained. At the present time, this property of the protein presents serious obstacles to its possible use in various applications.

At present, there are two problems in the recovery and use of protein from keratinous substances, namely, the burdensome and complex nature of the recovery procedure and the poor solubility of the recovered protein.

The present inventors, as a result of in-depth investigations aimed at eliminating these two problems, discovered that a protein extremely soluble in water and mixed solvents made up of water and hydrophilic organic solvents is obtained by a simple procedure, by cleaving disulfide bonds in keratin by reduction and then reacting it with a quaternary ammonium salt type cationizing agent, and thus arrived at the present invention.

Namely, the present invention relates to a process for producing modified keratin protein extremely soluble in water and mixed solvents made up of water and hydrophilic organic solvents, in which, in order to recover protein from a keratinous substance, the keratinous substance is subjected to reduction treatment with a reducing agent in water or a mixed solvent made up of water and a hydrophilic organic solvent to turn disulfide bonds (–S–S–) in the keratinous substance into sulfhydryl groups (–SH) and a cationizing agent having quaternary nitrogen and groups represented by [*See first two structures on page 205 – trans.*] or CH_2=CH– in one molecule is subsequently

added to the resulting sulfhydryl groups, or said sulfhydryl groups and other functional groups in the protein, for example, hydroxyl, amino, carboxyl, and other functional groups.

Below, the present invention is explained in detail.

Wool, feather, hair, nail, horn, etc. can be suggested as the keratinous substances used as the raw material in the present invention, but wool and feather are the most common materials.

Any reducing agents capable of cleaving the disulfide bonds of keratinous substances into sulfhydryl groups are suggested as the reducing agents used in the present invention. 2-mercaptoethanol, thioglycolic acid and other alcohols, or thiol derivatives of carboxylic acids, tri-*n*-butylphosphine, triphenylphosphine, and other phosphorus compounds, ascorbic acid and other organic reducing agents, as well as sodium hydrogensulfite, sodium sulfite, and other inorganic reducing agents can be used as the above-mentioned reducing agents.

The reaction of reduction of the keratinous substance in the present invention can be carried out by publicly known prior-art methods, but it is preferable to avoid introducing solubilization adjuvants such as guanidine sulfate and urea into the reaction medium. This is due to the fact that such solubilization adjuvants must be removed when protein is recovered upon termination of the entire reaction, which is a major factor contributing to the complexity of the protein recovery procedure. In other words, in the present invention, only water or a mixture of water and hydrophilic organic solvents in any proportion should be used as the reaction medium. Methanol, ethanol, *n*-propanol, isopropanol, acetone, methyl ethyl ketone, dioxane, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, hexamethylphosphoric triamide, etc. are suggested as examples of such hydrophilic organic solvents. Although there are no strict limitations on the amount of the reaction medium, the amount should be sufficient to conduct the reduction reaction in a uniform manner, the typically used amount being 10~100 times the amount of the keratinous substance by weight. The amount of the reducing agent used is generally in the range of from 2 to 10 times the equivalent required for the disulfide bonds in the keratinous substance used as the raw material. In addition, although there are no specific limitations on the pH of the reduction reaction system, it is necessary to select pH conditions, under which the keratinous substance does not substantially convert to oligopeptides, that is, typically in the range of from 2 to 12, and more preferably, 6 to 11.

Furthermore, as far as the reaction temperature is concerned, it is normally sufficient to conduct the reaction at room temperature. However, if necessary, it can be conducted under heating to shorten the time required for reduction. The reaction time has to be sufficient to bring the reaction to full completion and may vary depending on the reaction temperature, but usually the reaction requires 2 to 5 hours or more. In addition, it is preferable to conduct said reduction reaction in an atmosphere of an inert gas such as nitrogen. This is due to the fact that the sulfhydryl groups produced by the reduction reaction are very easily oxidized and are unstable to oxygen in the air.

Glycidyltrimethylammonium chloride, glycidyltriethylammonium chloride, 3-chloro-2-hydroxypropyltrimethylammonium chloride, allyltrimethylammonium chloride and corresponding bromides and iodides, etc. are suggested as the quaternary ammonium salt type cationizing agents used in the present invention, with glycidyltrimethylammonium chloride being the most commonly used agent.

The reaction of addition of the quaternary ammonium salt type cationizing agent to the reduction-treated keratinous substance used in the present invention is carried out by adding the quaternary ammonium salt type cationizing agent as is to the reduction reaction medium upon termination of the reduction reaction. The added quaternary ammonium salt type cationizing agent undergoes an addition reaction with the functional groups in the produced protein, primarily with the sulfhydryl groups. However, when added in excess of the equivalent required for the sulfhydryl

groups, it undergoes an addition reaction with functional groups other than the sulfhydryl groups, for example, such as hydroxyl, amino, carboxyl, and other groups. The appropriate amount of the added quaternary ammonium salt type cationizing agent is 0.1 to 6 times, and, more preferably, 0.5 to 2 times the equivalent required for the sulfhydryl groups produced by reduction in the keratinous substance. When it is less than 0.1, it is impossible to achieve sufficient solubility of the resulting modified keratin protein in water and mixed solvents made up of water and hydrophilic organic solvents, and when it is more than 6, the intrinsic properties of the protein are impaired, which is undesirable. In the range of from room temperature to 90°C, any temperature can be used as the reaction temperature, but the higher the temperature is, the more the addition reaction of the cationizing agent is promoted. Also, as concerns the pH of the system during the addition reaction, after the termination of the reduction reaction, there is no particular need to make pH adjustments. In other words, the reaction is conducted at a pH in the range of from 2 to 12, typically, in the range of from 6 to 11. As the addition reaction of the quaternary ammonium salt type cationizing agent with the reduction-treated keratinous substance proceeds, the keratinous substance dissolves in the reaction medium, with the final undissolved portion of it constituting 30% or less of the keratinous substance used as the raw material. The undissolved portion is removed by filtration, centrifugal separation, or other means, yielding a solution of modified keratin protein.

Upon removal of the reducing agent and other low molecular impurities from the thus obtained solution of modified keratin protein by means of ultrafiltration or dialysis, the solution can be used as is, but recovering the modified keratin protein in solid form by means of lyophilization is more convenient in terms of use as well as in terms of storage, transportation, etc.

Below, the present invention is illustrated specifically by referring to application examples.

Application Example 1

10g of wool fiber was immersed in 600g of an aqueous solution obtained by adding 0.02M Tris buffer and 6 ml of 2-mercaptoethanol was added thereto as a reducing agent, whereupon the pH was adjusted to 8.5 using 1N hydrochloric acid and a reduction reaction was conducted for 24 hours at room temperature in a stream of nitrogen. Next, 2.0g of glycidyltrimethylammonium chloride was added to the reaction system and the mixture was subjected to agitation for 6 hours at 50°C, solubilizing approximately 80% of the wool fiber in the reaction solution. The undissolved portion was removed by filtration while the reducing agent and other low molecular impurities were removed from the resulting aqueous solution of modified keratin protein by means of ultrafiltration (using a membrane with a molecular weight cut-off of 1,000), with the aqueous solution of modified keratin protein concentrated approximately 5 times in the process. 7.5g of modified keratin protein was obtained by lyophilizing it. The average molecular weight of the protein, obtained by gel filtration (using Sephadex G-75), was 41,000. In the following examples, the molecular weights were obtained in the same manner.

Application Example 2

7.8g of modified keratin protein with an average molecular weight of 40,000 was obtained by using the same procedure as in Application Example 1, with the exception of using a 50% aqueous solution of *n*-propanol as a reaction medium and 4 ml of tri-*n*-butylphosphine as a reducing agent.

Application Example 3

10g of wool fiber was immersed in 700g of a 30% aqueous solution of ethanol obtained by adding 0.02M Tris buffer and 4 ml of tri-*n*-butylphosphine was added thereto as a reducing agent, whereupon the pH was adjusted to 8.0 using 1N hydrochloric acid and a reduction reaction was conducted for 24 hours at room temperature in a stream of nitrogen. Next, 2.5g of allyltrimethylammonium chloride was added to the reaction system and the mixture was subjected to

agitation for 5 hours at 70°C, solubilizing approximately 85% of the wool fiber in the reaction solution. The undissolved portion was removed by filtration while the reducing agent and other low molecular impurities were removed by subjecting the resulting filtrate to ultrafiltration (using a membrane with a molecular weight cut-off of 1,000), with the filtrate concentrated to about 150 ml in the process. 8.2g of modified keratin protein with an average molecular weight of 39,000 was obtained by lyophilizing it.

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[ii] *This personal name may be also pronounced "Kaori," "Kaoru," "Iwao," and "Hajime." – trans.*

[iii] *This surname may be also pronounced "Furutani" or "Furudani." – trans.*